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The Inhibitory Effect of Vanadium Oxoanions on the Activity of Copper–Zinc Superoxide Dismutase

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ABSTRACT

The inhibitory effect of vanadate species on the enzymatic activity of bovine copper–zinc superoxide dismutase has been investigated at different pH values and vanadium concentrations. A definite inhibitory effect, clearly related to the main negative charge of each of the vanadate solutions, has been found. The results suggest that the origin of the inhibitory effect may be similar to that found for the phosphate ion, i.e., a diminution of the effectiveness of the substrate electronic guidance mechanism by partial neutralization of the charges close to the active site. Under physiological conditions, the inhibitory effect of vanadate is somewhat smaller than the phosphate.

Index Entries: Copper–zinc superoxide dismutase; vanadium oxoanions, inhibitory effect; effect of main negative charge; comparison with phosphate action.

INTRODUCTION

Bovine erythrocyte superoxide dismutase, EC 1.15.1.1 (SOD) is a dimeric enzyme containing both copper(II) and zinc(II) ions in each

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subunit (*1–5*). This metalloprotein is very efficient to prevent the accumulation of the toxic superoxide ion in tissues, by catalyzing the reaction

$$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$$

The site of reactivity for superoxide is the copper(II) ion, whereas the zinc(II) is of the structural type.

It had been reported by different authors that the presence of the phosphate anion can influence the properties of SOD. Recently, Mota de Freitas and Valentine (*6*) have demonstrated that the inhibitory effect of this oxoanion is primarily a result of the neutralization of the positive charge on the side chain of Arg–141, one of the residues proposed as responsible for the electrostatic guidance of superoxide to the active site (*7–10*).

As it is known, vanadate can compete with phosphate in many biochemical processes, because of their similarities in charge, protonation behavior, size, and geometry (*11,12*). For this reason some effects of the vanadate ion over the SOD activity can be expected. On the other hand, the very complex equilibria for the V/O-system at different pH values, generating different vanadate species, makes it possible to find new evidences on the suspected charge effects of oxoanions over this enzymatic system.

MATERIAL AND METHODS

Reagents

Superoxide dismutase, xanthine, xanthine–oxidase, nitro blue tetrazolium (NBT) and the employed buffers were obtained from Sigma; the sodium vanadate was obtained from Merck.

Stock solutions containing variable vanadium concentrations in the range between 10^{-2} and $10^{-5}M$ at different pH values (5.75 MES buffer; 6.2 MES buffer; 7.00 HEPES buffer; 8.00 TAPSO buffer; 10.75 CAPS buffer) were prepared by dissolving $NaVO_3$ in the corresponding $0.1M$ buffer solution.

The characterization of the vanadium oxoanions present in the different used solutions was achieved by electronic spectroscopy (*12,13*) and using the information from the well known equilibrium diagrams for the V/O systems as a function of pH and vanadium concentration (*11,13–15*).

Experimental Procedure

SOD activity was assayed by the method of Imanari et al. (*16*). This method is based on the inhibitory effect of SOD over the reduction of NBT by the superoxide anion generated by the system xanthine/xanthine–oxidase, measuring the absorption changes at 560 nm. Therefore,

it is possible to obtain a direct measurement of the variation of SOD activity as a consequence of the presence of the different vanadate forms.

Specific activity is expressed in terms of arbitrary SOD units; one SOD unit is defined as the quantity of the enzyme that would cause 50% inhibition of NBT reduction (*16*).

In all the investigated pH values and vanadium concentrations, control experiments with the system: buffered vanadate + NBT + (xanthine/Xanthine-oxidase), i.e., without SOD, were undertaken in order to exclude possible side effects on the vanadates over the generation of the O_2^- anion and/or on the coloration reaction of NBT.

RESULTS AND DISCUSSION

Characterization of the Vanadate Solutions

The analysis of the electronic spectra of the different prepared solutions, together with the information obtained from the equilibrium diagrams for the vanadate species as a function of pH and vanadium concentration, show the presence of the main anionic species presented in Table 1.

In spite of the complexity of these systems and equilibria, some important conclusions can be immediately obtained from the results shown in Table 1:

1. At a given vanadium concentration, the total amount of negative charge held by the anions diminishes with increasing pH values. This means, that at pH values between 5 and 6, the main negative charge of the present vanadate species may be around -5, at pH = 7–8 around -2.5 and at pH $\sim$10 around -2.
2. The condensation degree of the oxoanionic species diminishes in the same direction, i.e., it is practically negligible at the highest investigated pH value (10.75).
3. Although not clear to visualize, it is evident that at constant pH value the main negative charge of the system diminishes with the diminution of vanadium concentration.

Inhibitory Effect of the Vanadate Solutions

The results of our measurements clearly indicate an inhibitory effect of all the investigated vanadate solutions over the SOD activity.

We have chosen the results obtained at the two lowest vanadium concentrations, because the information in this range is more complete (cf. Table 1) and, on the other hand, these concentration values are also of greater biological relevance.

The general observed trend, is a diminution of enzymatic activity with pH diminution, at a constant vanadium concentration. Fig. 1

Table 1
Main Oxoanionic Species Present in the Prepared Buffered Vanadate Solutions.

V	PH 5.75	6.20	7.00	8.00	10.75
$10^{-2}M$	[a]	[a]	$V_3O_9^{3-}$	$V_3O_9^{3-}$	HVO_4^{2-} $HV_2O_7^{3-}$
$10^{-3}M$	[a]	$H_2V_{10}O_{28}^{4-}$	$V_3O_9^{3-}$ HVO_4^{2-}	$V_3O_9^{3-}$ HVO_4^{2-}	HVO_4^{2-}
$10^{-4}M$	$H_2V_{10}O_{28}^{4-}$ $V_{10}O_{28}^{6-}$ $HV_{10}O_{28}^{5-}$	$H_2V_{10}O_{28}^{4-}$ $V_{10}O_{28}^{6-}$ HVO_4^{2-} (?)	$V_3O_9^{3-}$ HVO_4^{2-} $H_2VO_4^-$ (?)	$V_3O_9^{3-}$ HVO_4^{2-} $H_2VO_4^-$ (?)	HVO_4^{2-} VO_4^{3-} (?)
$10^{-5}M$	$H_2V_{10}O_{28}^{4-}$ $V_{10}O_{28}^{6-}$ $HV_{10}O_{28}^{5-}$ (?)	$H_2V_{10}O_{28}^{4-}$ $V_{10}O_{28}^{6-}$ HVO_4^{2-} (?)	HVO_4^{2-} $H_2VO_4^-$ (?)	HVO_4^{2-}	HVO_4^{2-} VO_4^{3-} (?)

[a]Electronic spectra are difficult to measure, because of the strong coloration of the solution.

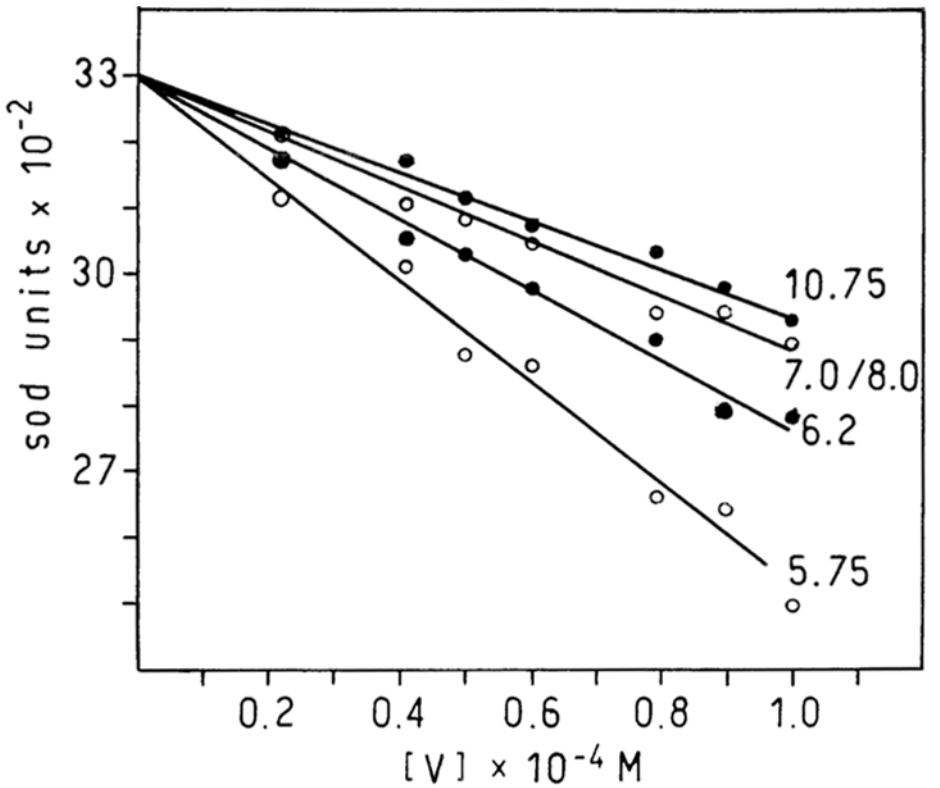

Fig. 1. Example of the inhibitory effect of vanadate solutions at different pH values.

shows this behavior in the vanadium concentration range between 0 and $10^{-4}M$.

It can also be observed from Fig. 1 that the inhibitory effects are practically the same at pH 7 and 8 and that the differences between pH 7/8 and 10.75 are less marked than those observed between pH 5.75 and 6.20.

A second trend is also clearly observed from Fig. 2, which shows that at constant pH values the inhibitory effect increases with increasing vanadium concentration.

The general conclusion which can be obtained from the results appearing in both figures are, at first sight, very simple: (1) At constant vanadium concentration a pH increase produces a diminution of the main negative charge of the anionic system and therefore the inhibitory effect is stronger on more acid pH values. This is also the reason for the greater differences seem in Fig. 1 for the pair of lines at pH 5.75 and 6.20 in comparison with the pair at 7/8 and 10.75, because the variation in negative charge, is smaller in the second than in the first case; and (2) At constant pH values the inhibitory effect is stronger at higher vanadium concentration, because of the same reason stated above, i.e., higher vanadium

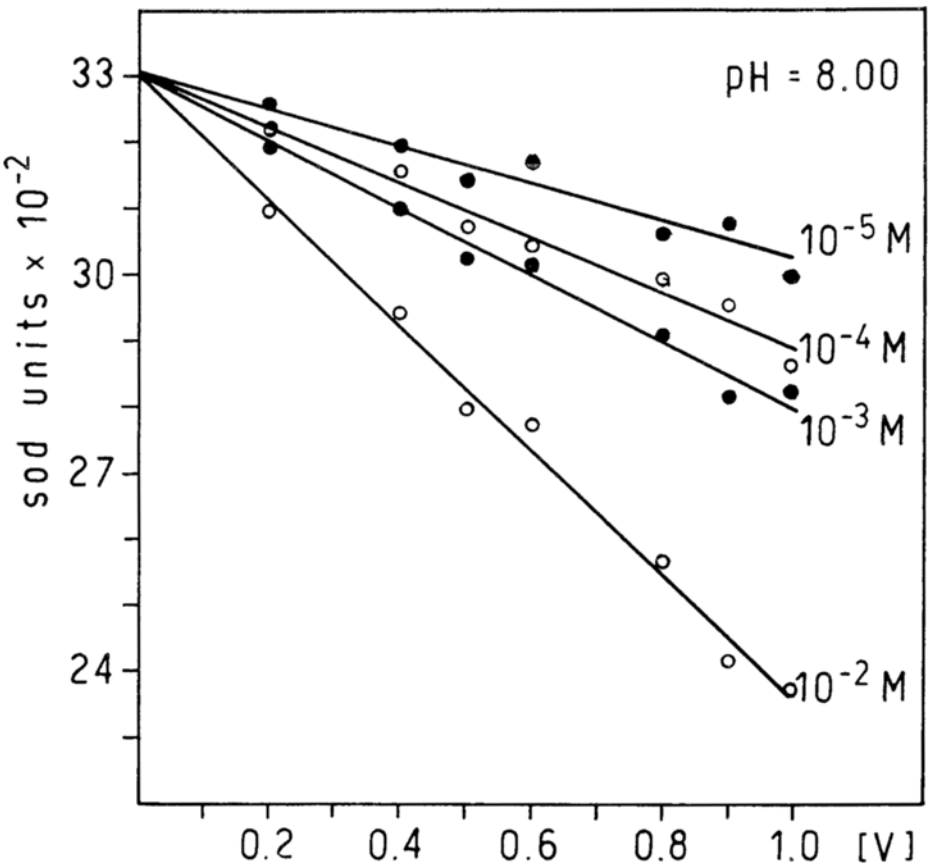

Fig. 2. Dependence of the inhibitory effect from the vanadium concentration at a constant pH value.

concentration implies higher main negative charge of the vanadate system.

Consequently, these results suggest that the electrostatic effect observed in the case of the phosphate anion operates also in these cases, pointing to a similar origin in the mechanism of inhibition for phosphate and vanadate species.

It is also possible that the ionic size of the anions contributes in some extent to the observed inhibitory effects (cf. for example (*17*)). As the degree of polymerization raises with the pH diminution, if the size effects were important, they would follow the same trend as the increase of the main negative charge. But such effect would be especially important at higher vanadium concentrations and therefore it can practically be excluded at biologically relevant concentrations.

We have also repeated experiences with phosphate solutions, under the same conditions employed for the vanadate experiments. The results at pH 7.00 and 10.75 show that the inhibitory effect of the phosphate ions is somewhat higher. This is the expected behavior, because under these

experimental conditions the negative charge of the phosphate species is always greater than that of the respective vanadate (PO_4^{3-} vs HVO_4^{2-} at pH ~11 and HPO_4^{2-} vs $H_2VO_4^-$ at neutral pH) (*11*). These results can be considered as an additional proof of the specific effect of the vanadium inhibition of SOD activity.

In conclusion, our study has shown that vanadate oxoanions produce a definite inhibitory effect on the SOD activity, with a mechanism probably similar to that known for the phosphate anion.

The facts that species with very different main negative charges are present in the investigated vanadate systems, and that the observed inhibitory effects are clearly related to these charges, can be considered as an important additional proof of the suggested charge neutralization mechanism (*6*).

Finally, these results constitute a new and interesting example of the similarities between some aspects of phosphorus and vanadium biochemistry.

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